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Regeneration of the Tentacles and Eyes of the Marine Snail Ilyanassa Obsoleta Stimpson

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REGENERATION OF THE TENTACLES AND EYES OF THE MARINE SNAIL
Ilyanassa obsoleta STIMPSON

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REGENERATION OF THE TENTACLES AND EYES OF THE MARINE SNAIL
ILYANASSA OBSOLETA STIMPSON

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The writer wishes to express his sincere appreciation to Dr. A. L. Applegate for his able direction of the problem and his helpful and timely criticism.

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INTRODUCTION

The capacity of invertebrates to renew lost parts has been verified through many experiments. Some of the most common examples of regeneration are found in platyhelminths, annelids, echinoderms and tunicates. In most of these animals a part or parts of the body have been cut or fragmented; and various degrees of regeneration have been observed (Hay, 1966; Lange, 1920; Lender, 1965; Hamburger, 1965; Huxley, 1911).

The Arthropoda exhibit the ability to regenerate lost appendages (Hay, 1966). In the crustaceans, structures such as antennae, thoracic legs, uropods, eyes, and chelae, have been removed and regeneration of these structures has been observed (Needham, 1965).

The removal of the eyestalks from an arthropod not only stimulates regeneration, but also has an effect on other physiological processes (Carlisle and Knowles, 1959). The removal of the optic stalk may result in regeneration of antennae instead of eyestalks (Needham, 1965; Przibram, 1934). The development of an antenna instead of an eyestalk is dependent upon how distal the amputation is made on the eyestalk. It was found that more distal amputations produced a normal eye and that more proximal amputations resulted in hetermorphism (Okada, 1944; Przibram, 1934).

In the Mollusca, regeneration has been observed in excised areas of the shell, tentacle, foot, and the head. Needham (1952) has demonstrated that regeneration can occur in a large portion of the foot of the gastropod, Harpa.

Regeneration in cephalopods has been observed in many specimens. Injured tentacles of the octopus and squid have been shown to regenerate. Lange (1920) demonstrated that the arms of the squid, when amputated, show complete regeneration. In the study by Lange, macroscopic as well as histological techniques were used to describe a step-by-step process of regeneration. The course of regeneration in the squid has been placed in three stages: the healing of the wound, the degeneration of the tissue, and the renewal of the structure (Lange, 1920).

Hamburger (1905) indicated that in gastropods the eye will regenerate, and other studies have shown that regeneration of the eye occurs in nudibranchs (Cucagna and Nusbaum, 1915). At the present, no histological study of nerve regeneration is Mollusca is available. Hanko (1913) does indicate that nerves which innervate the eye of Nassa mutabilia regenerate, but Hanko does not support his statement with a detailed study of neural regeneration.

Embryological studies have provided important information on the development of the eye. The eye of Haliotis originates as a group of pigmented epithelial cells on the outer base of each tentacle. The cells around the eye become raised on a small optic tubercle. A shallow retinal cup is formed by the invagination of the pigment cells. The rod cells of the retina differentiate and a crystalline lens is secreted in the opening of the retinal cup (Crofts, 1937). It has also been stated by Crofts that the cephalic tentacles develop from a small prominence in the pre-velar region. Eventually the tentacle elongates and becomes mobile.

In many gastropods embryological development is mosaic, a type of development in which removal of the cells from the embryo does not result in regeneration during the embryonic stages (Raven, 1958). Embryonic development of Ilyanassa is mosaic (Clement, 1962). Regulative embryonic development is evident in certain cephalopods such as the squid (Raven, 1958). It has been suggested that a correlation between regenerative powers and the regulative or mosaic type of development exists (Needham, 1952; Raven, 1958).

It was considered desirable to examine regeneration of the eye and tentacle of the marine snail, Ilyanassa obsoleta. The object of this study was to describe the detailed process of regeneration of the tentacles and eyes of Ilyanassa. The method used consisted of a step-by-step histological study of the epithelial, muscle, connective, and nerve tissues throughout the regenerative process.

The study consisted to two major steps: (1) A macroscopic study was undertaken to determine whether or not the eye and tentacle of a gastropod which exhibits a mosaic development pattern could regenerate. (2) A study was undertaken using histological techniques to determine the detailed step-by-step process of regeneration of the eye and tentacle in *Ilyanassa obsoleta*.

MATERIALS AND METHODS

Living Material

Shipments of the marine gastropod, Ilyanassa obsoleta, were received from the Woods Hole Biological Supply Facility in the Fall of 1966, in the Spring of 1967, the Summer of 1967, and in the Fall of 1967. All snails were maintained in a marine aquarium to provide the animals with a large volume (fifty gallons) of constantly circulating, aerated, and filtered salt water. The salt water was made by adding a commerical salt mixture, Instant Ocean (Jungle Laboratories, Orlando, Florida), to tap water until the specific gravity reached 0.0024. In order to maintain this level of salinity deionized water was added as necessary. The pH was maintained between 7.0 and 7.3 by adding either tribasic or dibasic sodium phosphate (1M).

The experimental animals and the control animals were placed into three small aquaria of approximately eight liters each. Two of the aquaria contained the experimental animals and one contained the controls (See Table I). The aquaria were interconnected by a linear siphon system, thus permitting continuous and uniform recirculation of aerated and filtered water. The three aquaria were maintained under natural light conditions. The over-all temperature range was kept within 70°-80°F.

Removal of Tentacles and Eyes

It was found that the use of relaxing drugs caused the animal to become anesthesized within its shell. This made the excision of the eye and tentacle impossible. The best method for cutting the tentacle and eye was found to result from placing the snail into a petri dish with sea water. When the animal extended its tentacles a razor blade could be brought down upon the area proximal to the tentacle and eye. (Figure 1). This method made it possible to accomplish the complete removal of the eye and tentacle.

The tentacle and eye of Ilyanassa obsoleta arise from a plate of tissue that is attached to the foot. Each tentacle was removed at its base which is attached to the plate of tissue (Figure 1). Both tentacles and eyes were removed from each experimental animal. After the operation, each animal was inspected under a dissecting microscope to assure that removal was complete. In some experiments, only one tentacle and eye was amputated to provide a more adequate comparison of regeneration between the experimental and control animals.

Histological Studies

During the investigation conducted in the Summer, 1967, histological sections were made of the tissue reforming on the second, fourth, seventh, ninth, twelfth and fourteenth days after amputation of the tentacles and eyes. During the investigations conducted during the Spring, 1968, tissue sections of the regenerating eye and tentacle were made at two-day intervals after amputation.

The tissues removed from the experimental animals were fixed in a solution formulated by Elftman (1959). After 24 hours, the fixed tissues were transferred to 70% ethyl alcohol. The tissues were then dehydrated through an ethyl alcohol series (30 min. per alcohol change) and then embedded in Paroplast (Preiser Scientific, Inc., Louisville, Kentucky; melting point 56-57°C). The embedded tissue was sectioned at 10 microns and mounted on a clean slide. The Aldehyde-Fuchsin-PAS method (Elftman, 1959) was used in staining the tissue from the Spring, 1967, and summer, 1967, groups. The tissue from the spring, 1968, experimental group was stained using a simplified Aldehyde-Fuchsin method (Cameron and Steel, 1959).

TABLE I

Date	Number of Animals	Type of Study
February through March, 1967	Fifteen; equally divided among three aquaria	Macroscopic
June and July 1967	Twenty-four; equally divided among three aquaria	Histological
July and August 1967	Twenty-four; equally divided among three aquaria	Histological
March, 1968	Twenty-four; equally divided among three aquaria	Histological

OBSERVATION AND RESULTS

Macroscopic

After the operation, bleeding was minimal with no apparent after effects. Following excision of the tentacle, no growth was observed until the fifth day (Figure 2). On the fifth day a small tip of white tissue was seen extending from the end of the stump (Figure 3). On the ninth day, the newly developing tip of tissue was longer and much more distinct compared to the fifth day (Figure 4). On the twelfth day the tentacles had an appearance which was essentially normal; they were smaller and of lighter color than the tentacles of the animals used as controls (Figure 5). The newly developed tentacles were narrower in diameter than the tentacles of the controls. By the eighteenth day the tentacles were hardly distinguishable from the controls. On the twentieth day, histological examination revealed the presence of an eye and a well developed tentacle.

Histological Studies

This study involved a more detailed account of the regeneration of the eye and tentacle in which several experimental groups were used so that regeneration could be observed at daily intervals after the amputation.

<u>Second Day</u> In the tissue sections studied, wound healing was

observed in which the area of the wound formed a dome-shaped structure over the raw tissue (Figure 6). Some degeneration of tissue was observed within the cap of tissue. Much of the tissue was irregular and consisted of broken macerated areas (Figure 7). In some sections the epithelium seemed to be growing over the degenerating tissue. This was thought to be the first blastema (Figure 8). Some sections showed the epithelial layer to be complete with an underlying tissue layer consisting of cells having relatively large nuclei; in other words, the deep layer appeared to be composed of blastic cells (Figure 9). In some cases nerve tracts could be observed passing toward the apex of the stump into the area of the blastic cells; in other cases the nerve tract was recessed from the region of the cut (Figure 10). The recessed nerve fibers appeared to be degenerate. Connective tissue and muscle tissue was also present in some parts of the blastic region, but there was no apparent organization in and around the cut area (Figure 6).

Pigment distribution among the cells of the epithelium was rather varied. Occasionally a pigment cell was seen in the blastic forming region (Figure 11).

Some difference was noted between the group investigated in 1967 and the group investigated in 1968. It was noticed that, in the group investigated in 1968, regeneration appeared to be more advanced in that the cap-shaped structure contained less degenerated tissue, and in that the pigmented cells appeared to be greater in number, especially in the epithelium (Figures 6 and 12).

<u>Fourth</u> <u>Day</u> The epithelium was more distinct consisting of columnar-shaped cells, having large nuceli and scattered pigment. In some sections the blastic region was rather small showing little indication of growth (Figure 13). In other sections the blastic layer was larger and appeared to be undergoing mitotic division.

Little degeneration of tissue was present, and it appeared that the damaged tissues had been repaired. In most sections the muscle and connective tissues were observed near any blastic cell mass that was present (Figure 14). Mucous cells were not present in the blastic area, but were found along the sides of the stump. Mucous cells were absent at the apex of the stump. In some sections squamous cells were noted in this blastic area, usually below the epithelium. Generally, nerve tracts that passed toward the tentacle were found in the areas of the old tissue, not near the blastic layer (Figure 15). In one section, however, the nerve tract was traced toward the cut area and out to the epithelium. In the epithelium a small eye was beginning to develop at the end of the nerve tract (Figure 16). The eye area was not within the blastic cell tissue but lateral to it. It appeared that the epithelium had invaginated toward the end of the nerve tract and that the epithelial tissue was being transformed into retinal tissue. The invaginated area was located at the base of the new tentacle-like projection. The epithelial cells, or now retinal-like cells, were becoming highly pigmented, especially within the cup-shaped invagination (Figure 17). The nerve was the optic nerve by definition and not a tentacular nerve.

Sixth Day The epithelial development varied from section to section. In most cases the columnar cells of the epithelium were longer and had more pigment than those found on the fourth day. The columnar cells of the epithelium appeared to merge into the blastic layer (Figure 11 and 18).

The blastic region had increased in size and was deeply stained. The new tentacle was longer in comparison to the four-day sections (Figure 19). The tentacles of the groups studied in 1968 were approximately 350 microns long. On the sixth day some vascular areas were evident at the bases of the new tentacles.

Nerve tracts could be seen near the general area of the blastic cells, but these tracts were not penetrating the blastic area (Figure 20). Connective and muscle tissues were present at the edge of the blastic cell area, but were not extended into the new tentacle.(Figure 19).

The eye was present, being more fully developed in that there was more invagination and the invaginated cells contained more pigment (Figure 17 and 21).

In the groups studied in 1967, the killing of the snails was extended one more day before sections were prepared. The tissue prepared on the seventh day in the study in 1967 was at approximately the same stage of regeneration as the six-day tentacles of the group studied in 1968; the only difference was that the tentacle was slightly longer and that the eye more invaginated and more pigmented.

Eighth Day The epithelium was more pigmented. Also it still had the same characteristics as described for the sixth day. The new tentacle was approximately 450 microns long in the 1968 group and was more vascular than before. These vascular areas occurred in the connective and muscle tissues. The blastic layer varied in size from section to section; and was located in the apex of the projecting tentacle. The blastic area was always located in the apex of the stump, with the connective and muscle tissues located behind the blastic layer (Figure 22).

The eye had developed more fully in that the invaginated sides had come together. In some sections, the eye was irregular in shape, e.g., elongated. The retinal cells were distinguishable from other cells and contained more pigment granules (Figure 23). Also a series of tiny droplets appeared at the free ends of the retinal cells (Figure 24). This secreted material had the same staining characteristics as the lens material in the eyes of the animals used as controls.

Tenth Day The tentacle had increased in length (750 microns). The epithelium had become better defined in some sections in that the nuclei and pigment could be seen. Individual cells were distinguishable. Pigment was increased in these epithelial cells. Connective and muscle tissues were present in the greater portion of the tentacle, and vascular cavities were present in greater numbers at the base of the new tentacle than at the apex (Figure 25). Nerve tracts appeared to be absent in the distal areas, but were found passing through the lower part of the new tentacle.

The cells of the blastic area appeared to be diminished in the surrounding regions compared to the eighth-day sections. The area of the blastic tissue was about the same size as the area found in eight-day sections, however, the blastic area appeared less dense than it was earlier.

The eye (approximately 75 microns in diameter) appeared complete, but was smaller than a normal (175 microns) eye. In some cases, the lens was missing; it was probably lost during the cutting of the sections. All structures of the eye were readily distinguishable and pigment concentration was higher than the eighth day (Figure 26). The eye appeared to have become separated from the outer epithelium by a thin membrane that was highly stained. This membrane appeared to surround the eye, separating it from the adjacent cells. Other invaginations around the area of the eye had now disappeared in most cases (Figure 26).

In the group investigated in 1967, sectioned on the eleventh day, the internal appearance of the eye and tentacle was the

the same as described in the 1968 group. The tentacle measured approximately 500 microns long.

Twelfth Day The epithelium was well developed along most of the length of the new tentacle, and pigment granules appeared to have increased in number compared to the ten day sections (Figure 27). The apex of the new tentacle still contained areas in which the difference between the blastic tissue and the columnar epithelium was indefinite (Figure 28). Connective and muscle tissues and vascular cavities were found throughout most of the length of the tentacle (Figure 29). The blastic area was dispersed in some sections. Blastic cells still could be found at the apex of the tentacle (Figure 30). Nerve tracts were present in the new tentacle, some extending most of the length of the tentacle.

The eye had increased in size (120 microns diameter) and contained greater amounts of pigment granules. A lens was present in some of the sections. The eye was smaller than the normal eye.

Pigment was present in the entire epithelial layer, and the epithelium was now more distinct from the rest of the cellular mass of the tentacle (Figure 29).

Fourteenth Day The tentacles (1200 microns long) were larger and longer than those found in the twelve-day sections. Connective and muscle tissues were present in the new tentacle except at the apex where the tissue was still essentially blastic. The blastic area was again more dispersed in appearance than in the ten-day sections. Probably some growth was still going on in the tentacle tip, as indicated by the presence of blastic cells (Figure 31). The nerve tracts were extended distally toward the blastic area (Figure 32). At this stage of regeneration most eyes were well developed, having retinal cells, lens, and pigment (Figure 33). The size of the eye was smaller than the normal eye. Also the eyes, when compared to a normal eye, were found no longer in an invagination but in a bulge of tissue known as an ocular bulge (Figure 33).

In summary, the first stage of regeneration involves wound healing in which the epithelium grows over the open wound. The epithelium constitutes a cap-like structure. Degeneration of damaged tissues such as connective, muscle, and nerve occurs. Next a blastic area develops following degeneration of damaged tissue in which connective, muscle, and nerve tissue seems to approach the blastic area without direct contact. An area between the blastic cells and the muscle and connective tissue consists of a zone of differentiation. Throughout the regeneration process epithelium grows outward from the stump forming a new tentacle-like structure. At the same time the optic nerve seems to initiate invagination of the epithelial tissue. The invaginated epithelium is transformed into retinal cells. In the cells of this invaginated area, pigment concentration begins to increase.

From this point on it appears that the blastic layer is the main point of growth, in that the connective, muscle, and nerve tissues all tend to differentiate behind the blastic layer. Next the blastic area appears to stop increasing in size. This occurs by the tenth day, after which the blastic cell mass appears to become less dense. The size of the blastic area slowly decreases in size to the fourteenth day. The eye continues to invaginate and close, while pigment concentration increases. The lens of the regenerating eye forms within the optic vesicle. Finally, after differentiation of muscle, connective, nerve, epithelial and eye tissues, the eye and tentacle become relatively complete except for size. Vascular cavities were present in muscle and connective tissues during the entire process of regeneration.

DISCUSSION

Wound healing which is nearly complete on the second day in Ilyanassa obsoleta appeared to be effected by a contraction of the wound area, followed by an overgrowth of epithelium. Lange (1920) showed in work preformed on squids in which an arm was removed, that the epithelium grows from surrounding epithelium, causing a contraction of the tissue around the stump of the arm. He further noted that before the epithelium began stretching over the wound, the membrane of uninjured epithelial cells adjacent to the wound drew back a little. Aftewards, the epithelium grew forward forming a cap over the cut area. The epithelial cells in Ilyanassa were irregular and varied in size and shape.

The blastic cell layer of the snail was present throughout most of the regeneration period but it could not be definitely indentified as the primordium of connective tissue, muscle tissue, or nervous tissue. The blastic layer remained consistant in area until the fourth day during which regeneration of the damaged structures began. Lange (1920) indicated that the first signs of regeneration of muscle tissue occurred when the blastic cells were noticed. These cells of the squid were termed sarcoblasts.

In most of the *Ilyanassa* tissue examined the muscle and connective tissue was near the blastic layer, which appeared in the stump or in the newly developed tentacle. This relationship was retained throughout most of the regeneration process. It has been indicated that new muscles are developed from sarcoblasts formed of the sarcoplasm and nucleus of the old muscles (Cucagna and Nusbaum, 1915). In the blastic area of the snail, *Ilyanassa*, perhaps there are also sarcoblast cells along with other types.

Melanophore development is of interest in the regeneration process in *Ilyanassa*. The pigmented cells occurred within the deep blastic cell layer, and as regeneration progressed, more and more of these cells were found in the outer epithelium. The data indicate that migration of cells occurred from the blastic area to the epithelium. It is possible that the epithelium developed from adjacent epithelium as indicated by the contraction of the cut area in earlier stages. The work of Lange (1920) indicates that in squid regeneration, new epithelial cells arise from the epithelium of the stump.

Usually in the amputated area or stump of *Ilyanassa* no nerve tracts may be found in the first few days after amputation. The absence of nerves could be due to disintegration or degeneration of the axial nerves. Because of the debris and macerated tissue of the two-day section, it would appear that disintegration of nerve tissue could have occurred in the stump. According to Lange (1920) the first signs of disintegration of the axial nerve is found in the larger ganglion cells. The only sign of degeneration in the squid neuropil is the shrinkage of the neuroglia nuclei.

During the period of eye regeneration, nerve endings probably degenerate to some degree and then regenerate. The regenerated nerve endings seem to initiate the invagination of the epithelium to form the eye primordium. Some work has been done on limb regeneration in certain amphibians. Nerves were removed from the limb bud and regeneration did not occur. When a nerve from another limb on the same animal was implanted in this area, regeneration resulted (Singer, 1950). A more recent study using limbs of opossum have also shown a regeneration-nerve relationship (Mizell, 1968). It is probable that the eye of Ilyanassa regenerates in essentially the same manner. It is clear that the part of the epithelium that invaginates does become a part of the eye. Also, it is apparent that there are several invaginations of which only one becomes the eye.

In the embryological studies by Crofts (1937), it was indicated that in the prosobranch embryo, eye development first appears in the form of a group of pigmented epithelial cells on the lateral side of the base of each tentacle, the cells becoming raised on a small optic tubercle. A shallow retinal cup was formed from the invaginating pigmented cells of epithelium. The rod cells of the retina differentiated and a crystalline lens was secreted, eventually blocking the opening of the retinal cup.

At one stage it was noticed that the inner cup of the eye has the same staining characteristic as the lens of the normal eye.

The stain was located in the areas of the free ends of the retinal cells. Therefore, it is indicated that possibly the lens was secreted from the retinal tissue.

In embryonic studies, Fretter and Graham (1962) found the cavity of the open vesicle of the eye usually contains a variable amount of secreted material, in some cases completely filling the cavity. This material possibly was the forming lens, although the true nature of the material is still doubtful. It appeared that the lens slowly developed from secreted material on the eighth day. The material appeared to originate from the retinal cells since some sections showed evidence of tiny droplets of lens-like material on the free ends of the retinal cells.

CONCLUSIONS

1. The process of regeneration of the eye and tentacle of _Ilyanassa_ can be described as occurring in three stages: (a) wound healing (b) blastema formation (c) redevelopment of the missing structures.

2. Cells of the blastic area apparently give rise to the epithelial, muscular, connective, and blood vascular tissues of the regenerating tentacle. Newly developed projections of the amputated nerve tracts in the stump supply the regenerating tentacle with neural elements. The epithelium of the new tentacle seems to be derived from both the blastic area and the epithelium of the stump.

3. Regeneration of the eye is evidently initiated by the presence, near the epithelium of the stump, of a newly-developed extension of the amputated optic nerve. The epithelium of the stump invaginates toward the nerve; the epithelial-lined invagination elongates, and it's blind end expands to form the optic vesicle. The optic vesicle is pinched-off from the surface epithelium as the epithelial cells lining the vesicle differentiate to form the retina.

4. The lens is formed from material secreted by the retinal cells.

SUMMARY

Macroscopic observations of Ilyanassa obsoleta, after removal of the eye and tentacle, prove that this marine gastropod is capable of regenerating the amputated structures.

Histological studies were undertaken to provide a detailed analysis of regeneration of the eye and tentacle. After amputation of the eye bearing appendages, regenerating tissue was removed from the amputee at daily intervals. The tissue was stained using the Aldehyde-Fuchsin techniques of Elftman (1959) and Cameron and Steele (1959). Connective, muscular, neural, blood vascular, and epithelial tissues in histological sections of ten microns thickness were studied.

Wound healing, the first event in the progress of regeneration, was found to be nearly complete after two days. A single layer of epithelial-like cells covered the area of maceration and degeneration at the site of amputation, and beneath this layer was an aggregation of blastic cells. The epithelial cells apparently are derived in part from the old epithelium of the stump and in part from the blastic layer.

Four days after amputation the blastic layer increased in size, pushing the epithelium outward from the stump; the resulting projection of tissue developed into the new tentacle.

at the same time the number of cells increased in the epithelium; these were probably derived from the blastic layer.

From the sixth day to the tenth day after amputation, the blastic area appeared highly active in the formation of the new tentacle. Connective, muscle and nerve tissues were located proximal to the blastic area as it moved outward at the apex of the regenerating tentacle; the connective and muscle tissues were more than likely differentiated from the blastic cells.

The nerve tracts of the regenerating tentacle grew outward from old nerve tracts in the stump. During the entire regeneration of the tentacle vascular sinuses were found in the connective and muscle tissue. The incised optic nerve in the stump grew outward toward the epithelium of the stump. The nerve evidently initiated an invagination of the stump epithelium. From the second day through the eighth day the invagination proceeded to become more elongated, more pigmented, and more expanded to form the optic vesicle. From the eighth through the fourteenth day the lens of the eye developed from a series of secretions from the retinal cells.

One of the most interesting aspects of the regeneration of the tentacles and eyes is that the structures of the tentacles are derived, for the most part, from the blastic area; the optic elements are formed from the unrenewed epithelium of the stump.

PLATE I

Figure 1. A drawing of Ilyanassa obsoleta before amputation. The shell has been cut away to show the tentacle and eye. The arrow and line indicate the area of excision.

Figure 2. A drawing of Ilyanassa after amputation.

Figure 3. First indications of growth after amputation. Fifth day.

PLATE I

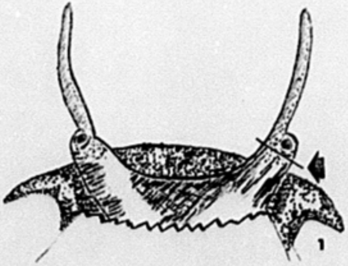

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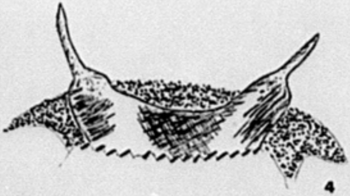
PLATE II

Figure 4. New tentacle growth after nine days.

Figure 5. Tentacles at the end of the twelfth day.



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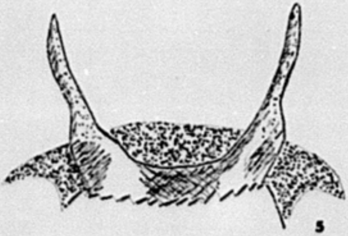

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PLATE III

Figure 6. Stump and cap-like structure after amputation. Second day. 130X magnification. Phasecontrast.

Figure 7. Macerated area in the stump. Second day. 550X magnification. Phasecontrast.

Figure 8. Epithelial cells at top of photograph. Arrows indicate blastic cells below epithelium. 550X magnification. Phasecontrast.

Figure 9. The epithelial cells and blastic cell layer. The arrows show the blastic cells. Second day. 550X magnification. Phasecontrast.

PLATE III

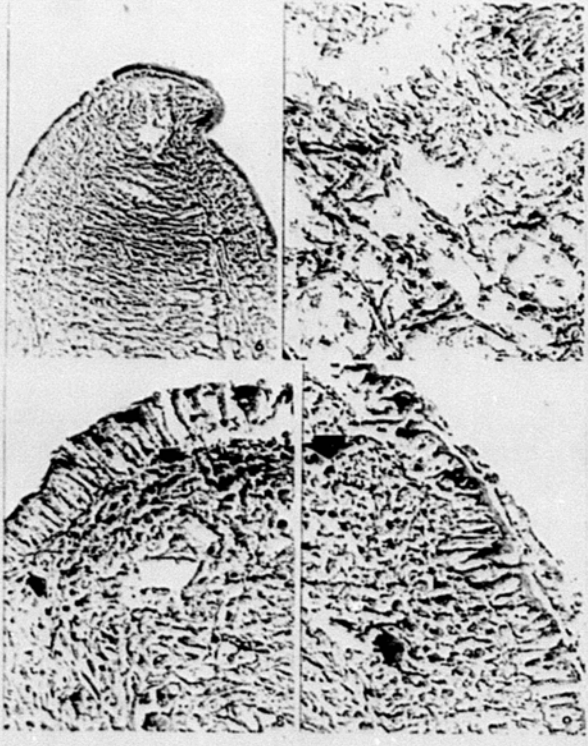


PLATE IV

Figure 10. Nerve tract (at arrow) in the stump. Second day. 130X magnification. Phase-contrast.

Figure 11. Pigment cells in the blastic area (at arrow) Note tiny granules. Second day. 550X magnification. Phasecontrast.

Figure 12. Spring 1968 group. The degree of development of the amputated area is shown. Second day. 130X magnification. Phasecontrast.

Figure 13. Spring 1968 group. Epithelial and blastic area (at arrows) development is shown. Fourth day. 550X magnification. Phasecontrast.

PLATE IV

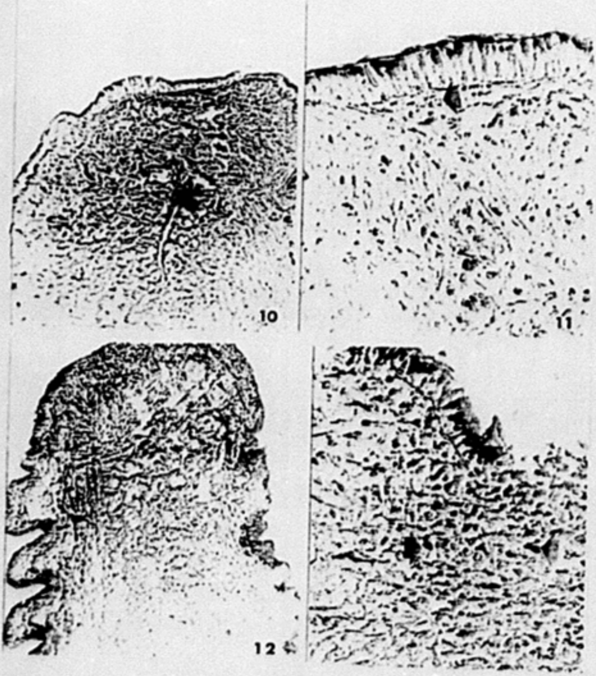


PLATE V

Figure 14. New tentacle containing deeply stained blastic cells. Fourth day. 130X magnification. Phasecontrast.

Figure 15. A nerve tract (at arrow). Fourth day. 130X magnification. Phasecontrast.

Figure 16. The eye and a nerve tract. The nerve is located below the eye on the photograph. Arrows show nerve tract. Fourth day. 130X magnification. Phasecontrast.

Figure 17. First indication of the eye as an invagination. Fourth day. 550X magnification. Phasecontrast.

Figure 18. Epithelium and blastic area. Fourth day. 550X magnification. Phasecontrast.

PLATE V

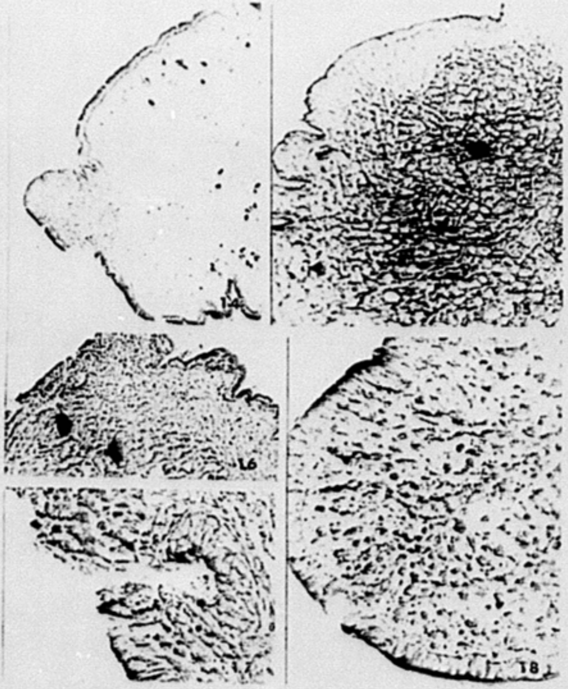


PLATE VI

Figure 19. Connective and muscle tissue in the stump. The new tentacle appears as a lobe of tissue at the left of the photograph. 130X magnification. Phasecontrast.

Figure 20. Nerve tract (at arrow) in the stump region. The new tentacle is at the top of the photograph. 130X magnification. Phasecontrast.

Figure 21. Continued development of the eye (at arrow). Sixth day. 550X magnification. Phasecontrast.

Figure 22. Differentiation of the connective and muscle tissue as shown on the sixth day. The eye is to the right of this photograph. Sixth day. 130X magnification. Phasecontrast.

PLATE VI

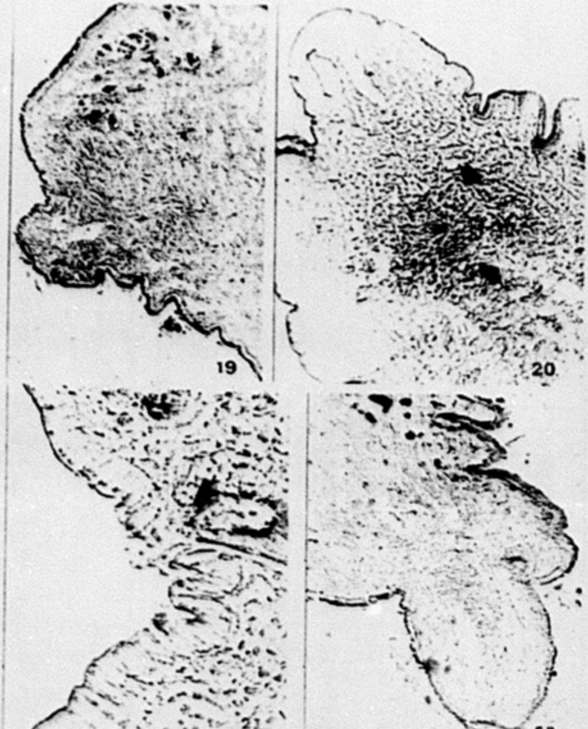


PLATE VII

Figure 23. Elongation of the invagination of the eye. Arrow indicates the invagination. Eighth day. 130X magnification. Phasecontrast.

Figure 24. The arrow marks the position of lens-forming droplets inside the eye. Eighth day. 550X magnification. Phasecontrast.

Figure 25. Tentacle growth with vascular sinuses. Tenth day. 130X magnification. Phase-contrast.

Figure 26. Optic bulge containing the eye with a lens. Tenth day. 130X magnification. Phasecontrast.

PLATE VII

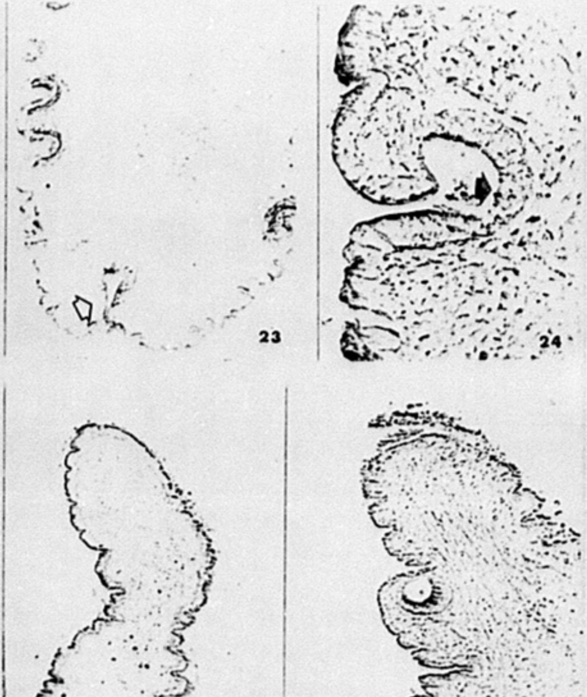


PLATE VIII

Figure 27. Increase of pigment in the epithelium. Blastic cells are found below the epithelium. Twelfth day. 550X magnification. Phasecontrast.

Figure 28. Indistinctiveness of seperation of epithelial and blastic layers. Twelfth day. 550X magnification. Phasecontrast.

Figure 29. Connective and muscle tissues with sinus cavities in tentacles. 130X magnification. Phasecontrast.

Figure 30. The blastic area in the apex of the tentacle. Twelfth day. 130X magnification. Phasecontrast.

PLATE VIII

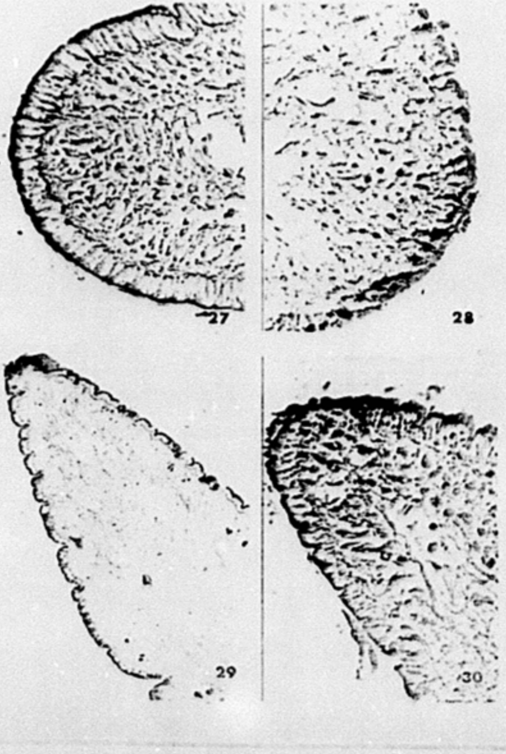


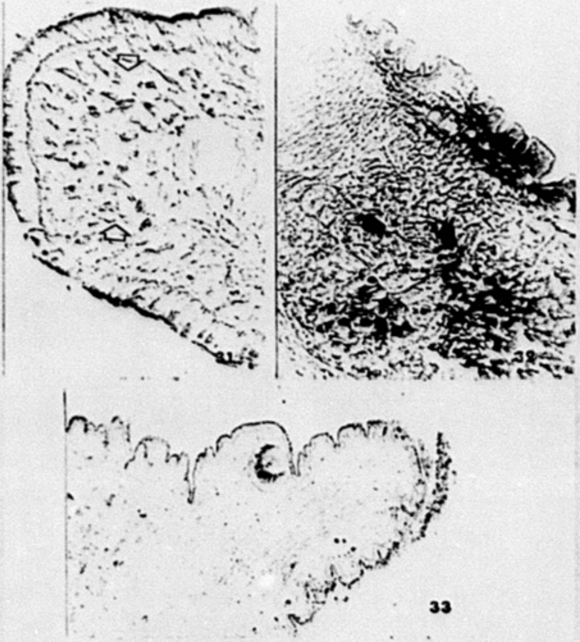
PLATE IX

Figure 31. Cell density of blastic layer at the apex of the tentacle. Fourteenth day. 550X magnification. Phasecontrast.

Figure 32. Nerve tracts (at arrow) in the stump. Fourteenth day. 550X magnification. Phasecontrast.

Figure 33. Complete eye having lens, retinal cells, and pigment cells. Fourteenth day. 130X magnification. Phasecontrast.

PLATE IX



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